(12) UK Patent Application (19) GB (11) 2 148 122 A

(43) Application published 30 May 1985

(21) Application No 8424147

(22) Date of filing 25 Sep 1984

(30) Priority data

(31) 537687 (32) 30 Sep 1983 (33) US

(71) Applicant
Dr. El Gendler,
917 S Shenandoah, Los Angeles, California 90035,
United States of America

(72) Inventor
Dr. El Gendler

(74) Agent and/or address for service
McNeight & Lawrence, Regent House, Heaton Lane,
Stockport, SK4 1BS

(51) INT CL⁴
A61F 2/28

(52) Domestic classification
A5R AP

(56) Documents cited

GB A 2068734		GB	1340451
GB A 2002240		US	4294753
GB	1525667	US	4172128
GB	1453243	US	3458397

(58) Field of search
A5R

(54) Process for stimulating induction of bone formation and stimulation of bone regeneration by artificially perforated bone matrix

(57) This invention is related to a process of encouraging induction of bone formation and stimulation of bone regeneration by bone matrix. The invention involves forming a plurality of perforations in a bone or bone matrix (14) prior to the implantation of same. These perforations facilitate the ingrowth of cells and blood vessels after the implantation of bone matrix and produce a significant increase in the ability of bone matrix to induce bone formation and stimulate bone regeneration.

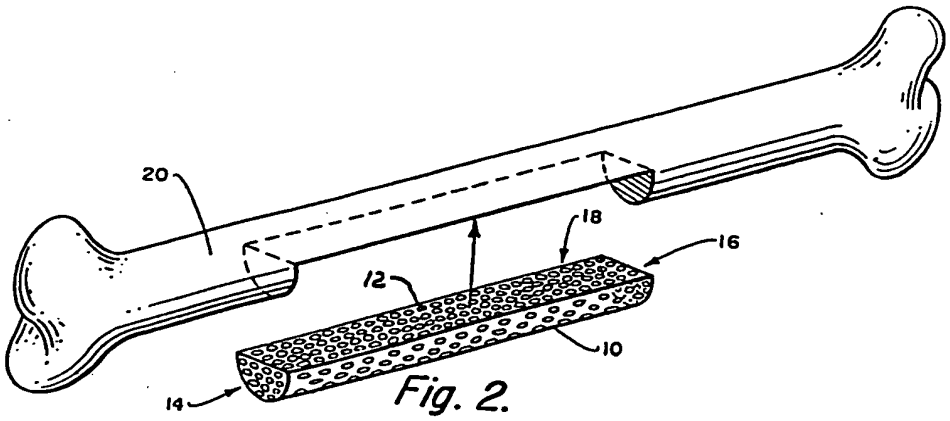

Fig. 2.

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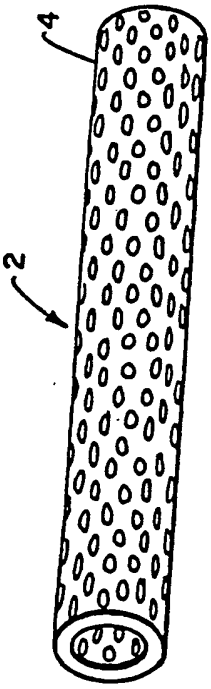

Fig. 1.

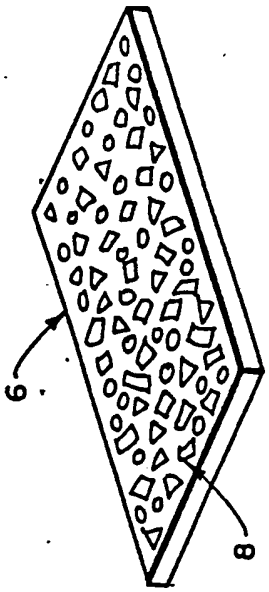

Fig. 1b.

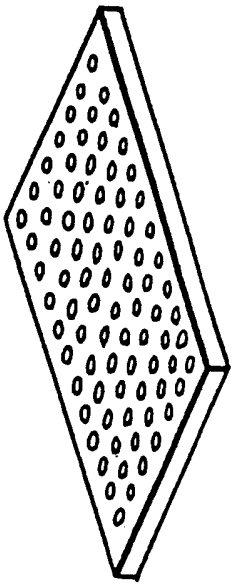

Fig. 1a.

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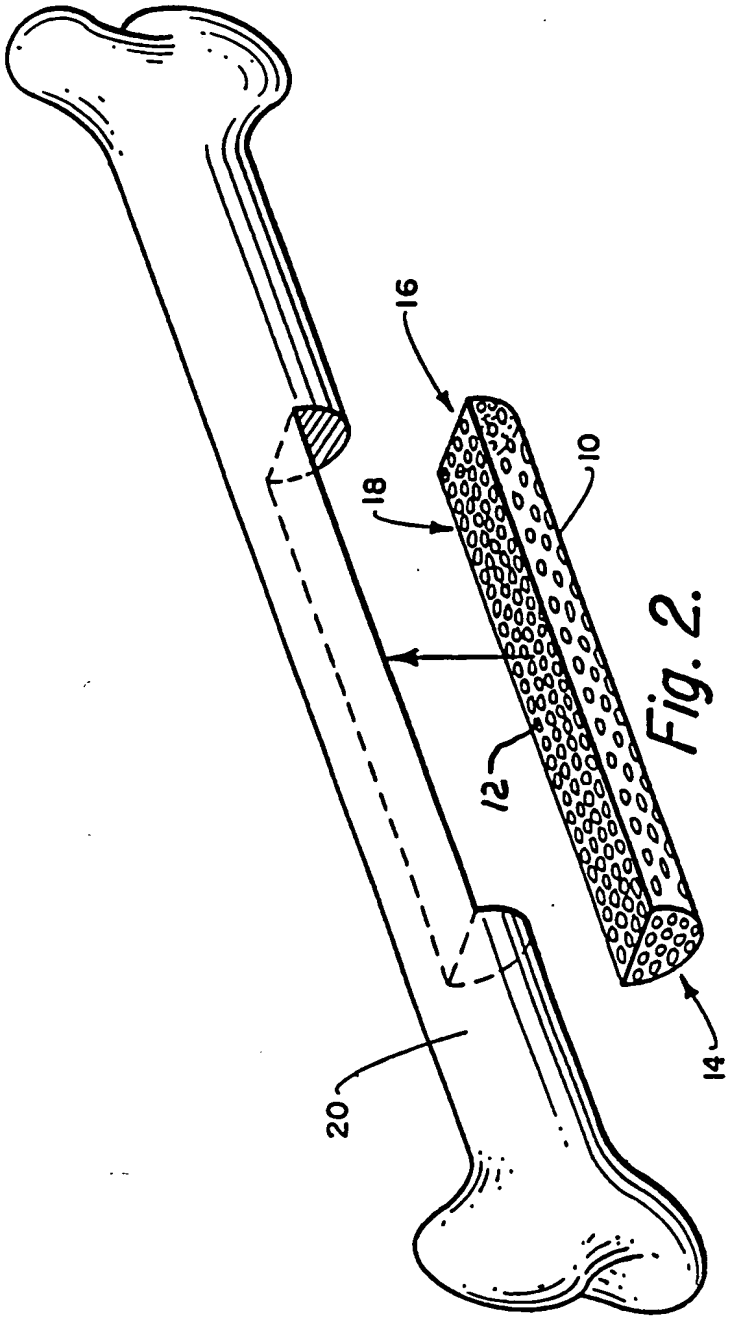

Fig. 2.

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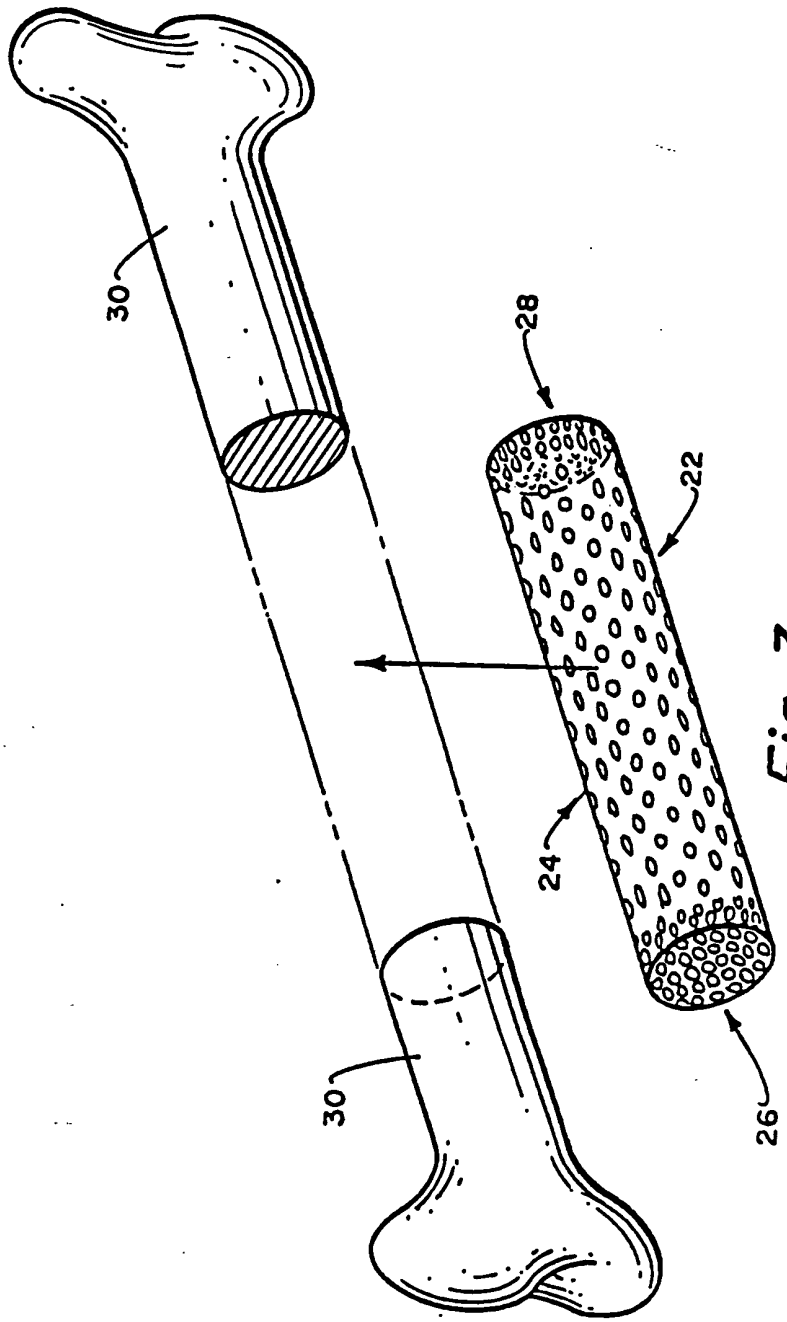

Fig. 3.

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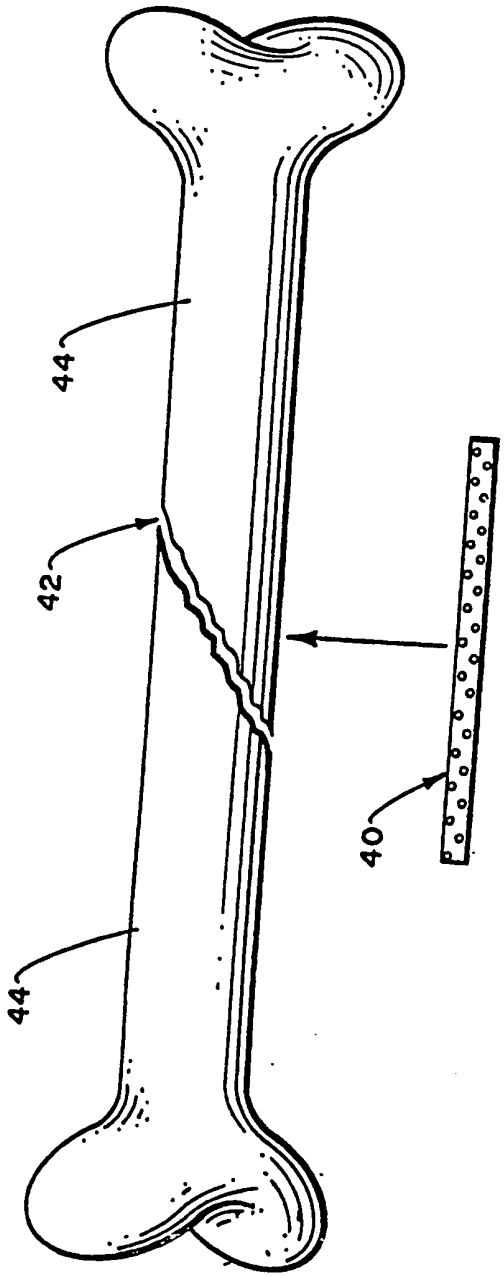

Fig. 4.

SPECIFICATION

Process for Stimulating Induction of Bone Formation and Stimulation of Bone Regeneration by Artificially Perforated Bone Matrix

The present invention relates to a process to further stimulate and encourage reconstruction, regeneration and ingrowth of bone, tooth and cartlidge material and more particularly to a process of preparing bone matrix by forming artificial perforations therein which is useful in surgery and dentistry and which is similar in its structure and composition to natural bones and teeth, to a process of orthopedic reconstruction using the artificially perforated bone matrix taught herein, and to products, such as the artificially perforated bone matrix, obtained by the process taught herein. The invention involves forming multiple artificial perforations in bone or bone matrix material prior to its surgical implantation in order to facilitate ingrowth of cells and blood vessels after the implantation of same and to produce a significant increase in the ability of the implanted material to induce bone and cartlidge formation, and to stimulate bone regeneration.

As is known, bones and teeth are composed of a matrix of organic material consisting of collagenous fibrils and a binding substance of mucopolysaccharides as well as of the inorganic component, namely calcium phosphate in the form of hydroxyapatite. The organic matrix is formed by filiform molecules arranged parallel to each other. Furthermore, the tissue is transversed by numerous microscopic capillaries which are oriented in various directions to said filiform molecules.

It is known that if the inorganic component is partially or completely removed from the bone or tooth, the remaining organic bone material, called bone matrix, can be transplanted to other living animal or human bodies without substantial deleterious effects. Consequently, bone matrix is used in modern medical procedures for its ability to stimulate and encourage reconstruction, regeneration and ingrowth of bone tissue after its implantation into a body site.

While the prior art has recognized the need for methods and material to stimulate and encourage reconstruction, regeneration and ingrowth of bone, none have disclosed the unique process of the herein disclosed invention.

Myers, et al. Patent No. 3 458 397 is directed to a process for producing osteogenic material from animal bone tissue. The osteogenic material is injected into an animal for the purpose of inducing bone formation. In this process, the bone is comminuted with pepsin in an acid solution and then digested, extracted and precipitated. There is no suggestion in this reference, however, of artificially perforating the bone matrix as is taught herein.

Urist Patent No. 4 294 753 is directed to a bone morphogenetic protein process for separating proteins from bone tissue. As in the previous reference, this reference calls for a comminution of the bone and the demineralization of the bone tissue. The demineralized bone tissue is then treated in an aqueous solution with a water soluble, neutral salt and a solubilizing agent. The neutral salt and the solubilizing agent are then separated, and the bone morphogenetic protein is precipitated. Once again, no mention of any type of perforation in the produced substance is shown.

Sano Patent No. 2 621 145 is directed to bone mat compositions and includes particulates of bone which are then enmeshed in a fibrin network. This produces a bone mat which is supported on a carrier strip stated to be of a flexible, plastic material. The process taught in this reference produces a flexible strip for use in bone surgery and promoting the regrowth of bone and includes what is termed a plurality of unboiled particles of ground, whole bone enmeshed in a fibrin network. This reference does not provide for perforating the bone mat composition or fibrin network as is taught herein.

Rapkin Patent No. 2 968 593 describes a method of preparing inorganic bone material by heating animal bone material in a liquid to a temperature from about 80°C. to about 100°C., drying the heated bone material, substantially defattening it with a fat-extracting solvent, and removing the organic matrix from the defattened bone material, for instance, by extraction with ethylene diamine to obtain the inorganic matrix. Such an inorganic bone material which is free of organic matter is used for transplantation from an animal of one species to another species without any adverse effect. However, there is no provision taught in the reference for perforating the inorganic bone material so produced.

Thiele, et al. Patent No. 4 172 128 is directed to a process of degrading/regenerating bone and tooth material. This reference provides a method of making a bone material which is implanted into an area to stimulate bone growth. In this reference the bone material is first ground and then the organic matrix of the bone is demineralized. A colloidal solution of the organic matrix is formed and ions are caused to diffuse into the colloidal solution in order to form a gel. Although the substance created through this process appears to be used for osteogenesis, this reference does not provide for perforating said substance as is taught herein.

It is one object of the invention to provide a novel process of producing an artificially perforated bone matrix to encourage bone ingrowth formation and stimulation of bone regeneration when the perforated bone matrix is implanted in orthopedic reconstruction procedures by forming a plurality of artificial perforations in the bone matrix, the artificially perforated bone matrix being readily accepted by the body and corresponding in its composition and structure to natural bones and teeth.

Another object of the present invention is to provide such novel and valuable artificially perforated bone matrix useful in the reconstruction and regeneration of natural bones and teeth.

Yet another object of the invention is a novel process of orthopedic reconstruction of skeletal

structure using the artificially perforated bone matrix produced by the novel process taught herein.

Other objects of the present invention and advantageous features thereof will become apparent as the description proceeds.

In principle, in a process of bone matrix preparation for surgical implantation, the improvement of the present invention comprises adding a step of forming a plurality of artificial perforations in the bone matrix prior to surgical implantation of same by a surgeon.

Another embodiment of the invention is the artificially perforated bone matrix produced in accordance with the process of the invention taught herein.

Still another embodiment of the invention is a novel process of using artificially perforated bone matrix to encourage bone ingrowth formation and stimulation of bone regeneration by surgically implanting the artificially perforated bone matrix in orthopedic reconstruction procedures.

Figure 1 is a side elevational view of a bone matrix having a plurality of artificial perforations therein;

Figure 1a is a side elevational view of an alternately formed bone matrix having a plurality of artificial perforations therein;

Figure 1b is a side elevational view of an alternately formed bone matrix having a plurality of artificial perforations therein of varying sizes and shapes;

Figure 2 is a side elevational view of an artificially perforated bone matrix and a bone having a cavity type defect therein, showing how the artificially perforated bone matrix can be used in tamponade or closing the bone defect or cavity;

Figure 3 is a side elevational view showing a bone having a gap defect therein and an artificially perforated bone matrix being positioned to span the bone gap defect; and

Figure 4 is a side elevational view showing a bone having a fracture defect therein and an artificially perforated bone matrix being positioned to induce bone induction and regeneration to close the fracture defect.

Referring to the figures of drawings wherein like numbers of reference designate like elements throughout, the present invention is directed to an improvement in the process for the preparation of bone matrix for use in surgical procedures which comprises adding a step of forming a plurality of artificial perforations in said bone, bone material, or bone matrix prior to the implantation of same.

Bone matrix may be produced using any one of the known prior art methods which may include any combination of the following steps.

A bone or bone material is harvested from any of the vertebrates. It can then be conserved by any of the known conservation methods. Partial or complete demineralization of the bone or bone material is carried out to cause decalcification by subjecting the bone or bone material to treatment with different acids, chelating agents, electrolysis or any combination of the foregoing. Finally, either prior to or after the demineralization of the bone, bone material or bone matrix, fixation and different physical and chemical processing is done. The prepared bone, bone material or bone matrix 2, shown in Figure 1, is then processed to form a plurality of artificial perforations 4 therein.

The plurality of perforations 4 may be formed in bone matrix 2 by drilling, laser, puncture or the like process. Perforations 4 may be of various shapes, such as, but not limited to, circular, triangular, multi-angled, irregular, slit-like or any combination of the foregoing.

The number of perforations 4 in bone matrix 2 may vary. Multiple perforations, however, produce a substantial increase in the ability of the perforated bone matrix to induce bone formation or stimulate bone regeneration that appears to be, within a specified range, proportional to the number, size and placement of individual perforations in the bone matrix.

Neither must the perforations be of a uniform size or shape in the bone matrix. Figure 1b shows an alternately shaped bone matrix 6 having a plurality of varying shaped and sized perforations 8 therein. It has been noted, however, that perforations having a maximum cross sectional area of 0.25 mm to 1.0 mm are optimally sized perforations to induce a substantial increase in the ability of the artificially perforated bone matrix to induce bone formation or stimulate bone regeneration.

Similarly, the perforations need not be uniformly concentrated over the surface area of the bone matrix. In fact, a higher concentration of perforations in a given area of the bone matrix will cause greater stimulation of bone growth or regeneration in that particular area over other areas having a lower concentration of perforations. This result is particularly useful in orthopedic procedures where the artificially perforated bone matrix abuts existing bone as in tamponade or closing of any bone defect or cavity, or in procedures to span gaps in bones.

More particularly, Figure 2 shows a tamponade situation wherein a bone matrix 10 has a plurality of artificial perforations 12 which are concentrated on sides 14, 16 and 18 of bone matrix 10 abutting bone 20. In bone matrix 10 intermediate sides 14 and 16, there is a lesser concentration of perforations 12.

Similarly, in surgical procedures involving bridging bone gaps, shown in Figure 3, a bone matrix 22 is shown having a plurality of artificial perforations 24 concentrated on and adjacent to sides 26 and 28 while intermediate sides 26 and 28 the perforations are less concentrated. Bone 30 is also shown.

Figure 4 shows how a portion of perforated bone matrix 40 is used in treating situations involving a bone fracture 42 in bone 44.

In these or similar cases, a higher concentration of perforations adjacent to, or in the sides of the bone matrix abutting the existing bone structure will cause greater bone formation, ingrowth and regeneration in these more perforated areas of the bone matrix than in other less highly perforated areas. Thus, with increased bone formation, ingrowth or regeneration in these abutting areas, the perforated bone matrix will more quickly adhere

to existing bone structure to close the bone defect, cavity or gap in a secure manner.

The perforated bone matrix produced as taught herein, can be used in surgery for treatment of acute bone fractures, and old non-healing bone fractures. Perforated bone matrix can also be used for tamponade or closing of any bone defect or cavity. The following specific examples serve to illustrate the present invention without, however, limiting the same thereto.

EXAMPLE ONE

A laboratory-controlled test was performed to examine the induction of bone formation by implantation of perforated bone matrix in laboratory test animals. A perforated bone matrix was prepared in accordance with the invention taught herein and was implanted subcutaneously lateral to the sternal edge of each laboratory test rat chosen to form a test group.

Four days after the implantation procedures were accomplished, a sample of laboratory rats from the test group were prepared and samples taken of the implantation site. Under microscopic examination, it was found that inside the perforations of the implanted bone matrix there was an accumulation of newly formed undifferentiated cells with a high activity of alkaline phosphatase in their cytoplasm.

Seven days after the implantation was accomplished, a further sample of laboratory rats from the test group were prepared and samples taken of the implantation site. Under microscopic examination, it was found that there were a small number of differentiated chondroid cells and fibroblasts among the young undifferentiated cells filling the perforations. Alkaline phosphatase activity was noted as being increased compared to the previous observation taken at four days.

Two weeks after the implantation was accomplished, another sample of laboratory rats from the test group was prepared and samples taken of the implantation site. Under microscopic examination, it was found that some matrix resorbtion around the edge of the perforations with a replacement of the bone matrix material by chondroid cells and osteoblasts had occurred. The perforations were filled with newly formed bone trabeculas covered with osteoblasts. The travecular bone was interspaced with islands of chondroid tissue and newly formed blood vessels. Also, the alkaline phosphatase activity in the cells filling the perforations was very high.

One month after the implantation was accomplished, a sample of laboratory rats from the test group was prepared and samples taken of the implantation site. Under microscopic examination it was found that the main part of the implanted perforated bone matrix had undergone resorbtion and had been replaced by newly formed trabecular bone interspaced with occasional islands of chondroid tissue. Newly formed trabecular bone was not restricted to previously implanted perforated bone matrix, but was seen beyond this area and surrounded with a capsule resembling the periosteum.

EXAMPLE TWO

This test examined the stimulation of bone regeneration by the implantation of perforated bone matrix. Several laboratory rabbits were chosen to form a test group. The rabbits each had a piece of ulnar bone approximately 1.5 to 2 cm removed from the mid-shaft of the ulnar bone and a fragment of perforated bone matrix prepared in accordance with the procedure taught herein inserted into the defect. Control x-rays taken immediately following the implantation procedure clearly showed the bone defect.

One week after the implantation, x-ray examination of the implantation site still showed the defect in the ulnar bone clearly. A sample of rabbits from the test group were prepared and samples taken in the implantation site. Under microscopic examination it was found that all the perforations of the implanted perforated bone matrix were filled with young undifferentiated cells with high activity of alkaline phophatase.

Two weeks afer the implantation, x-ray examination showed a small amount of mineralization in the area of implanted perforated bone matrix. A sample of rabbits taken from the test group were prepared and samples taken of the implantation site. Under microscopic analysis it was found that all perforations were filled with chondroid cells interspaced with newly formed bone trabeculas.

One month after the implantation, x-ray examination showed that the implanted perforated bone matrix had undergone mineralization. A sample of rabbits from the test group was prepared and samples taken of the implantation site. Under microscopic analysis it was found that the implanted perforated bone matrix had undergone resorbtion which had been spreading from centers established around the perforations. It was also found that the implanted bone matrix was being replaced by newly formed trabecular bone which was connected with the trabecular bone growing from the ends of the bone fragments.

Two months after the implantation, x-ray examination showed that the defect in the ulnar bone of the test rabbits had been replaced by highly mineralized bone tissue. A final sampling of rabbits from the test group was prepared and samples taken of the implantation site. Under microscopic analysis is was found that the ulnar bone defect had now been filled with bone undergoing remodeling.

EXAMPLE THREE

This test examined the induction of bone formation by perforated bone matrix having different sized perforations. A bone matrix was prepared as in Example One, in accordance with the procedure taught herein, with perforations of different diameter: 0.25 mm, 0.35 mm, 0.5 mm, 0.75 mm, 1.0 mm, 1.25 mm, 1.5 mm, and 2.0 mm. The perforated bone matrix was implanted subcutaneously in laboratory rats as outlined in Example One. At different times following the implantation, a sample of laboratory rats from the test group was prepared and samples taken of the

implantation site. Microscopic analysis of the implanted bone matrix showed that the most active process of bone formation was seen in specimens having a perforation diameter of 0.25 to 0.5 mm. In specimens with perforation diameter of 0.75 to 1.0 mm osteogenic processes were somewhat less active and in specimens with perforation diameter of 1.25 to 2.0 mm osteogenesis was even lower than that previously observed above.

EXAMPLE FOUR

This test examined the induction of bone formation by perforated bone matrix with a different density of perforation.

Several test groups were established each containing a number of laboratory rats. Perforated bone matrix was prepared as described herein and subcutaneously implanted in each laboratory rat in each respective test group.

In the first group perforated bone matrix had been prepared with perforations 0.25 mm in diameter. There were twenty-five perforations per one square centimeter (cm^2). The calculated sum of the perforated surface was 1.23% of the total surface area of the perforated bone matrix. Under microscopic analysis this perforated bone matrix showed high osteogenic activity.

In the second group perforated bone matrix had been prepared with perforations 0.35 mm in diameter. There were approximately twenty perforations per one square centimeter. The calculated sum of the perforated surface was 1.92% of the total surface area of the perforated bone matrix. Under microscopic analysis this perforated bone matrix showed high osteogenic activity.

In the third group bone matrix had been prepared with perforations 0.5 mm in diameter. There were approximately sixteen perforations per one square centimeter. The calculated sum of the perforated surface was 3.14% of the total surface area of the perforated bone matrix. Under microscopic analysis this perforated bone matrix demonstrated high osteogenic activity.

In test group four bone matrix had been prepared with perforations 0.75 mm in diameter. There were approximately twelve perforations per one square centimeter. The calculated sum of the perforated surface was 5.3% of the total surface area of the perforated bone matrix. Under microscopic analysis this perforated bone matrix showed osteogenic activity which was somewhat lower than in the previous three test groups.

In test group five bone matrix had been prepared with perforations 1.0 mm in diameter. There were approximately eight perforations per one square centimeter. The calculated sum of the perforated surface was 6.28% of the total surface area of the perforated bone matrix. Under microscopic analysis this perforated bone matrix showed osteogenic activity which was also somewhat lower than in test groups one through three.

In test group six bone matrix had been prepared with perforations 1.25 mm in diameter. There were approximately six perforations per one square centimeter. The calculated sum of the perforated surface was 7.36% of the total surface area of the perforated bone matrix. Under microscopic analysis, this bone matrix showed osteogenic activity which was considerably lower than that found in test groups one through five.

In test group seven bone matrix had been prepared with perforations 1.5 mm in diameter. There were approximately four perforations per one square centimeter. The calculated sum of the perforated surface was 7.06% of the total surface area of the perforated bone matrix. Under microscopic analysis, this perforated bone matrix showed osteogenic activity which was considerably lower than that found in test groups one through five.

In test group eight bone matrix had been prepared with perforations 2.0 mm in diameter. There were approximately three perforations per one square centimeter. The calculated sum of the perforated surface was 9.42% of the total surface area of the perforated bone matrix. Under microscopic analysis, this bone matrix showed very low osteogenic activity.

The invention described above is, of course, susceptible of many variations and modifications, all of which are within the skill of the art. It should be understood that all of such variations and modifications are within the spirit and scope of the invention and of the appended claims. Similarly, it will be understood that it is intended to cover all changes and modifications of the examples of the invention herein disclosed for the purpose of illustration which do not constitute departures from the spirit and scope of the invention.

CLAIMS

1. In a method of bone matrix preparation for surgical implantation, the improvement which comprises: forming a plurality of artificial perforations in said bone matrix prior to surgical implantation of same.

2. The improvement in accordance with claim 1 wherein said perforations are regular in cross-section.

3. The improvement in accordance with claim 1 wherein said perforations are irregular in cross-section.

4. The improvement in accordance with claim 1 wherein said perforations are uniformly distributed throughout said bone matrix.

5. The improvement in accordance with claim 1 wherein said perforations are concentrated in at least one area of said bone matrix.

6. A bone matrix having a plurality of artificial perforations formed therein in accordance with the process of claim 1.

7. The improvement in accordance with claim 1 wherein said perforations are triangular in shape.

8. The improvement in accordance with claim 1 wherein said perforations are polygonal in shape.

9. The improvement in accordance with claim 1 wherein said perforations are irregular in shape.

10. The improvement in accordance with claim 1 wherein said perforations are slot-like in shape.

11. The improvement in accordance with claim 1 wherein said perforations are circular in shape.

12. The improvement in accordance with claim 1 wherein said perforations are formed by drilling.

13. The improvement in accordance with claim 1 wherein said perforations are formed by puncture.

14. The improvement in accordance with claim 1 wherein said perforations are formed using a laser.

Printed in the United Kingdom for Her Majesty's Stationery Office, Demand No. 8818935, 5/1985. Contractor's Code No. 6378.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.